

2/30

FIG. 1 con't

5/30

Earl
SapI
BmrI

Earl

AlwNI
Bsu36I
BbvCI
Bpu10I
BamHI

703 (SEQ ID NO. 5)

← R1 Primer

CTHNPSSSS (SEQ ID NO. 6)

5/30

FIG. 3 con't

6/30

300

450

60

150

210

270

330

390

450

510

570

630

690

750

810

870

930

990

1050

1110

1170

1230

1290

1350

1410

1470

1530

1590

1650

1710

1770

1830

1890

1950

2010

2070

2130

2190

2250

2310

2370

2430

2490

2550

2610

2670

2730

2790

2850

2910

2970

3030

3090

3150

3210

3270

3330

3390

3450

3510

3570

3630

3690

3750

3810

3870

3930

3990

4050

4110

4170

4230

4290

4350

4410

4470

4530

4590

4650

4710

4770

4830

4890

4950

5010

5070

5130

5190

5250

5310

5370

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6090

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6810

6870

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6990

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7170

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7830

7890

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8010

8070

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10710

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10950

11010

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11130

11190

11250

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11790

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11970

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12090

12150

12210

12270

12330

12390

12450

12510

12570

12630

12690

12750

12810

12870

12930

12990

13050

13110

13170

13230

13290

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13410

13470

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13710

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13830

13890

13950

14010

14070

14130

14190

14250

14310

14370

14430

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14850

14910

14970

15030

15090

15150

15210

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15450

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15810

15870

15930

15990

16050

16110

16170

16230

16290

16350

16410

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16590

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16950

17010

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17190

17250

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17370

17430

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18090

18150

18210

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18330

18390

18450

18510

18570

18630

18690

18750

18810

18870

18930

18990

19050

19110

19170

19230

19290

19350

19410

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19890

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20010

20070

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20190

20250

20310

20370

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21210

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21390

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21510

21570

21630

21690

21750

21810

21870

21930

21990

22050

22110

22170

22230

22290

22350

22410

22470

22530

22590

22650

22710

22770

22830

22890

22950

23010

23070

23130

23190

23250

23310

23370

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23970

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24090

24150

24210

24270

24330

24390

24450

24510

24570

24630

24690

24750

24810

24870

24930

24990

25050

25110

25170

25230

25290

25350

25410

25470

25530

25

FIG. 4

Ear1
 Sap1
 Ear1
 5'-GAGGAGAGATACACCCCTCTGTTANGAATTCGAATGCAAGAGCTTCGATAATGGAATGAACAGCATGATAGAAATGCCGACTTTTCAATGTTTACCTTACCTTGAAGGAGAGAGGAATCAATCAACCCCAAGTCACAGTGAATC
 600
 AACTCCCTTCTATGTGGGAGACAATACCTTTAACTTTACCTTCGAACTATTACCTTACTTGTGTAATCTTACGGCTGAACACTTACAAATGGATGGAACTTCCTTCTCTTCTTAGTTAGTGTGGGTTCACATGCACCTTAG
 7/30
 ← R2-HA Primer
 E E D T P S V M E I E M E E L D K W M N S M N R N A D F E C L P T L K E E K E S N H N P S D S E S
 Bsu36l Bpu10l BamHI
 ATCCATATGATGTTCCAGATATGCTTAAGGATCC
 637 (SEQ ID NO. 7)
 TGGTATACACAGGTCTAATACGATTCCTAGG
 R2-HA Primer
 P Y D V P D Y A
 HA-Taq

FIG. 4 *con't*

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FACs Analysis of Transfected 293-EBNA Cells

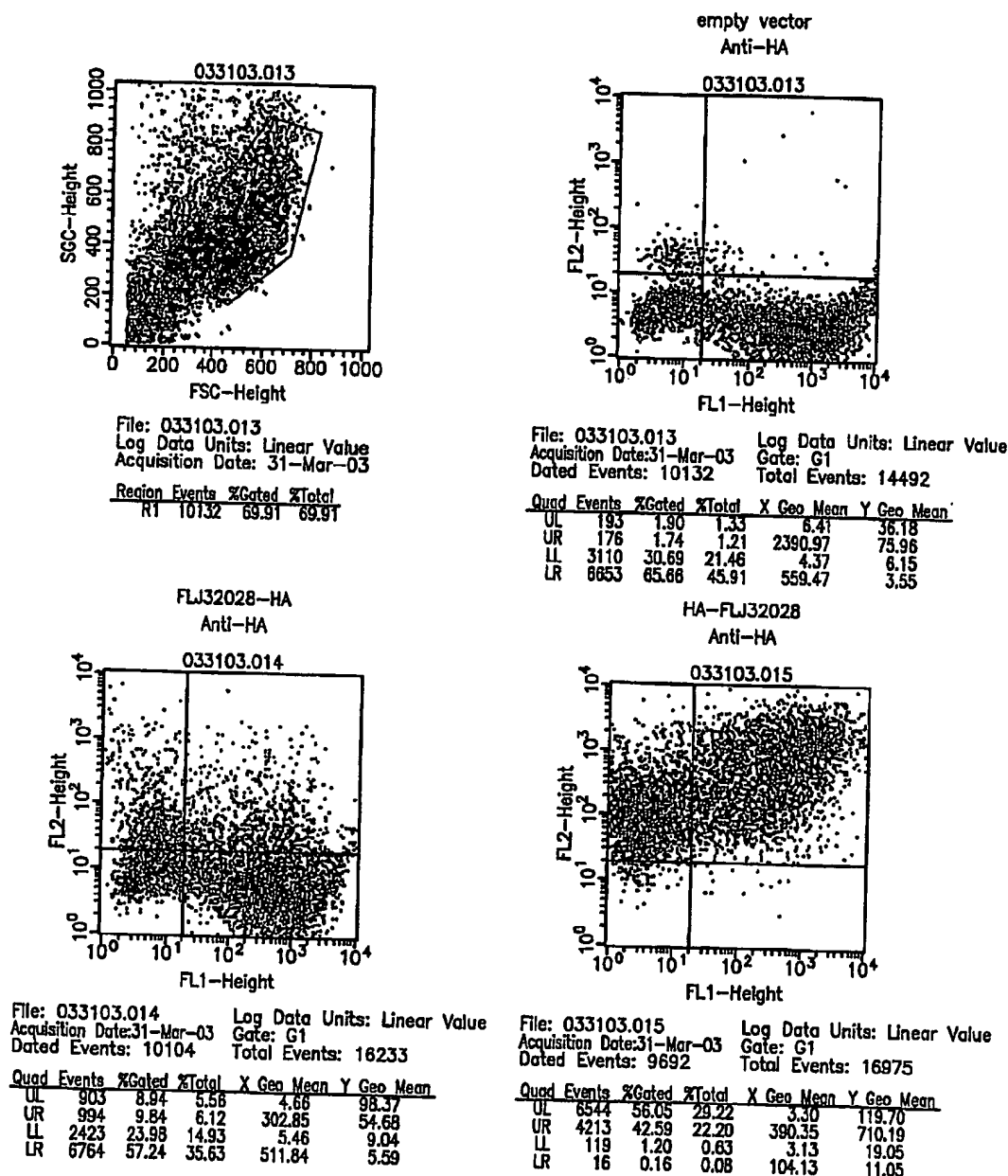


FIG. 5

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Anti-HA Western Blot of Transfected 293-EBNA Cells

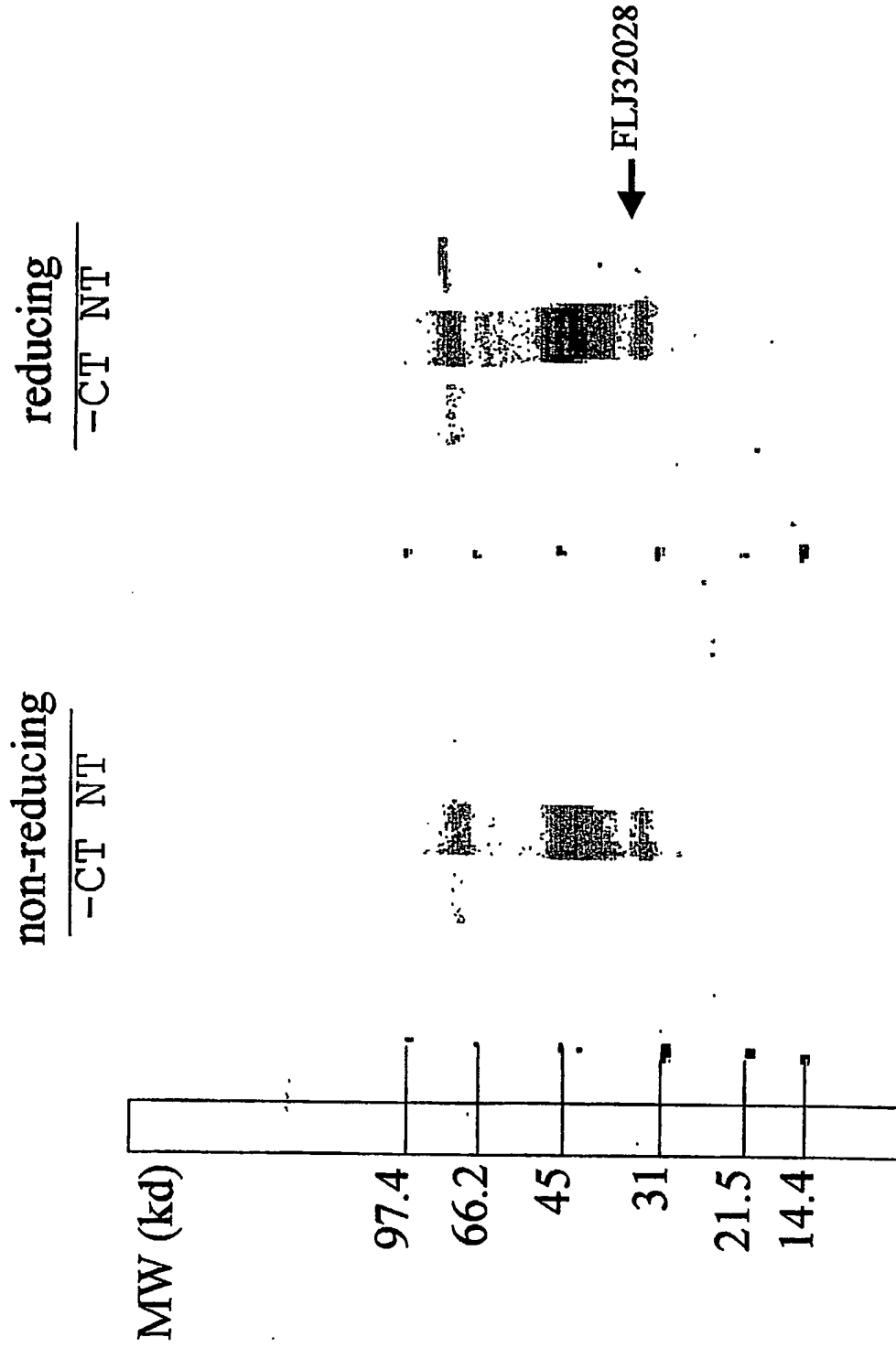


FIG. 6

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FLJ32028 polynucleotide sequence (SEQ. ID No. 2):

AGCCCGGCGCAGCATCCTGAGCGCGCCTCTGCCGAGGCGAGCGGACATG
CAGGCTCCCCGCGCAGCCCTAGTCTTCGCCCTGGTGATCGCGCTCGTTCC
CGTCGGCCGGGGTAATTATGAGGAATTAGAAAACCTCAGGAGATACAACTGT
GGAATCTGAAAGACCAAATAAAGTGACTATTCCAAGCACATTTGCTGCAGTG
ACCATCAAAGAAACATTAAATGCAAATATAAATTCTACCAACTTTGCTCCGGA
TGAAAATCAGTTAGAGTTTATACTGATGGTGTTAATCCCATTGATTTTATTGG
TCCTCTTACTTTTATCCGTGGTATTCTTGCAACATACTATAAAAGAAAAAGA
ACTAACAAGAACCTTCTAGCCAAGGATCTCAGAGTGCTTTACAGACATATGA
ACTGGGAAGTGAAAACGTGAAAGTCCCTATTTTTGAGGAAGATACACCCTCT
GTTATGGAAATTGAAATGGAAGAGCTTGATAAATGGATGAACAGCATGAATA
GAAATGCCGACTTTGAATGTTTACCTACCTTGAAGGAAGAGAAGGAATCAAA
TCACAACCCAAGTGACAGTGAATCCTAAACCTGAATGGCGCTCATGTTTTCC
AAGAGAAGCAGCCCCTGAGGGAGTCTGCTGAGGCTGCCAACA

FIG. 7

FLJ32028 polypeptide sequence (SEQ. ID No. 1):

MQAPRAALVFALVIALVPVGRGNYEELNSGDTTVESERPKNKVTIPSTFAAVTIK
TLNANINSTNFAPDENQLEFILMVLIPLILLVLLLSVFLATYYKRKRTKQEPSSQ
GSQSALQTYELGSENVKVPIFEEDTPSVMEIEMEELDKWMNSMNRNADFELP
TLKEEKESNHNPSDSES

FIG. 8

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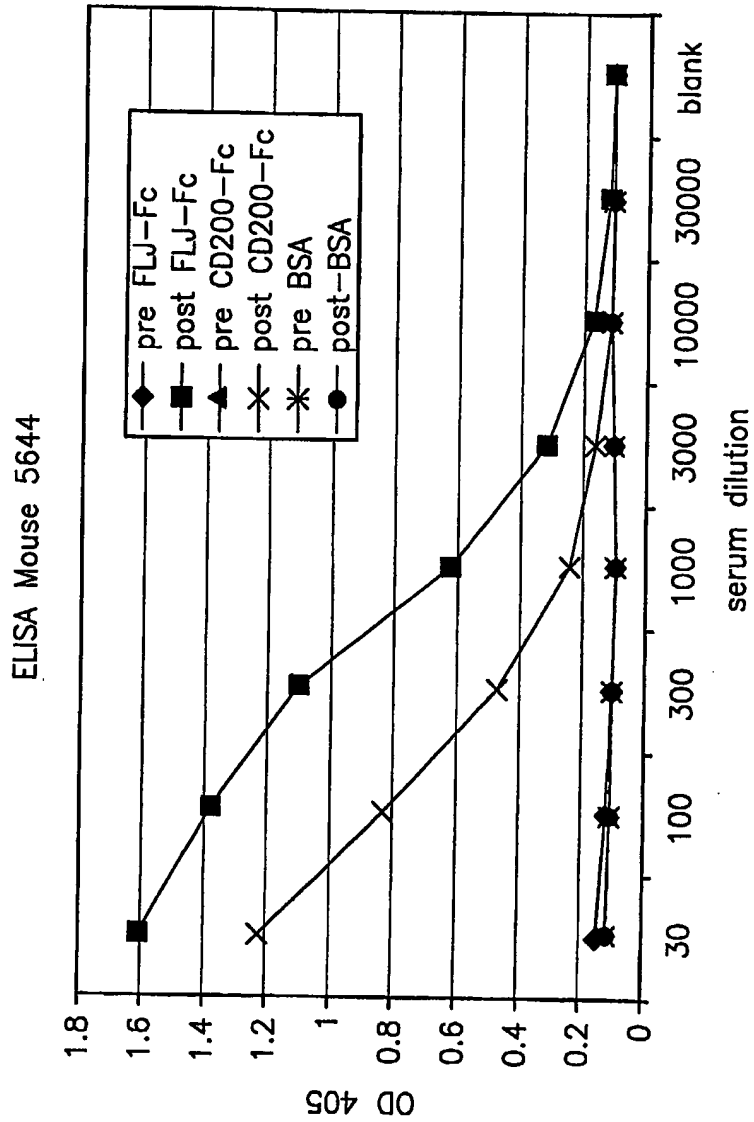
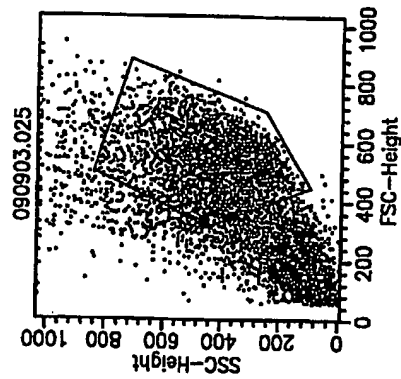


FIG. 9A

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Mouse 5644



Key	Name	Parameter	Gate
▨	090903.025	FL2-H	G1 pre 1:30/FLJ
—	090903.031	FL2-H	G1 post 1:30/FLJ
—	090903.037	FL2-H	G1 post 1:100/FLJ
—	090903.043	FL2-H	G1 post 1:300/FLJ
—	090903.007	FL2-H	G1 post 1:30/mock
—	090903.013	FL2-H	G1 post 1:100/mock
—	090903.019	FL2-H	G1 post 1:300/mock

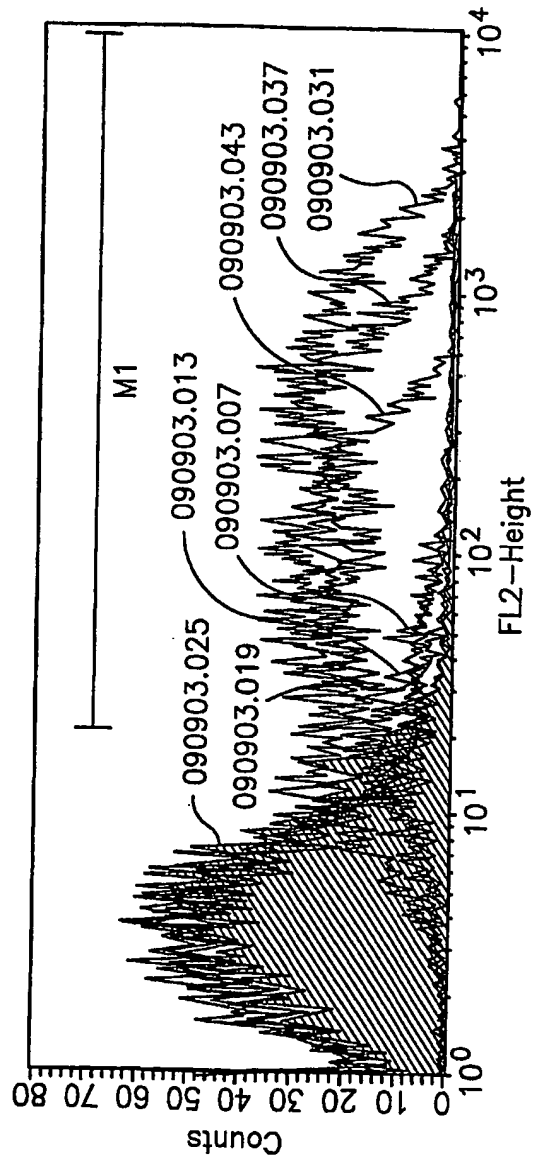


FIG. 9B

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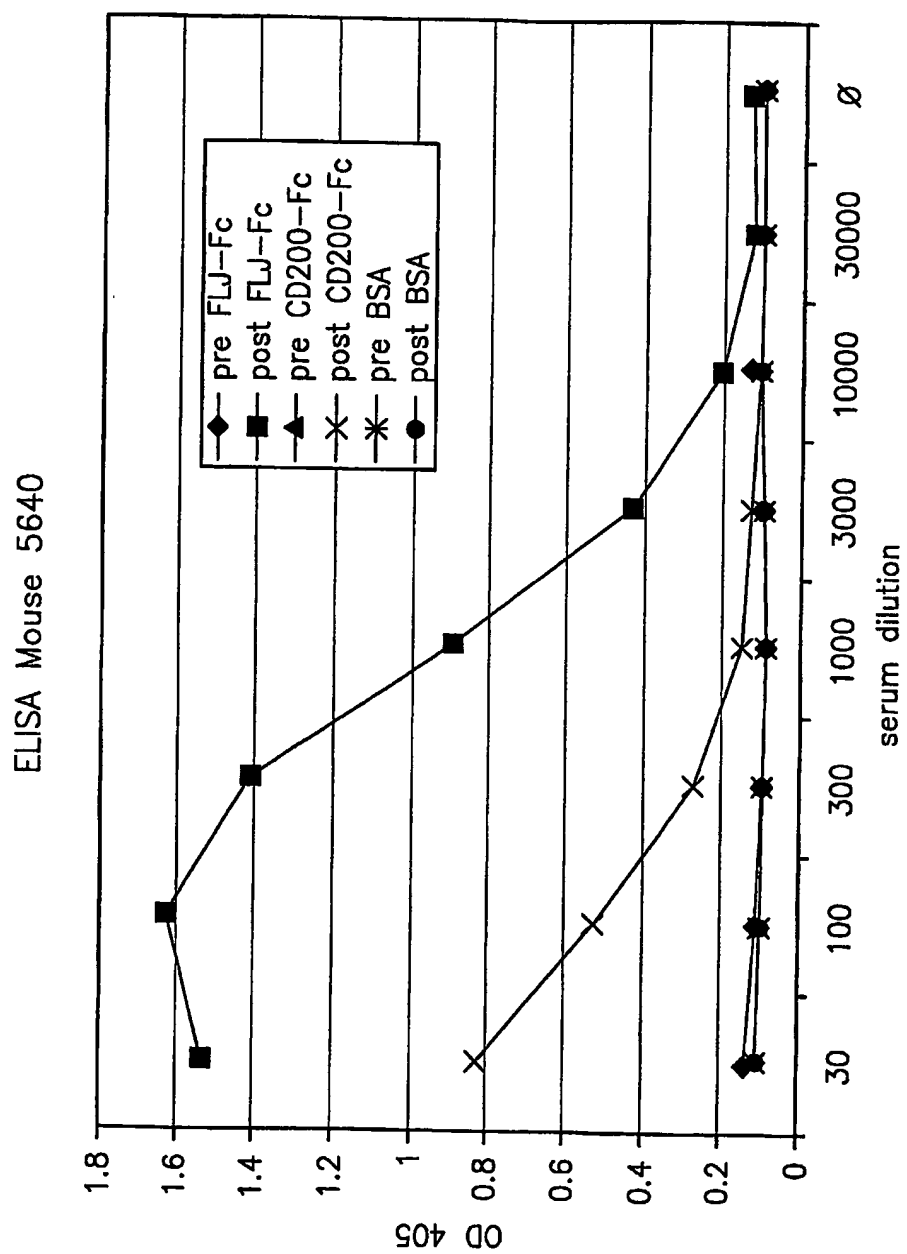


FIG. 10A

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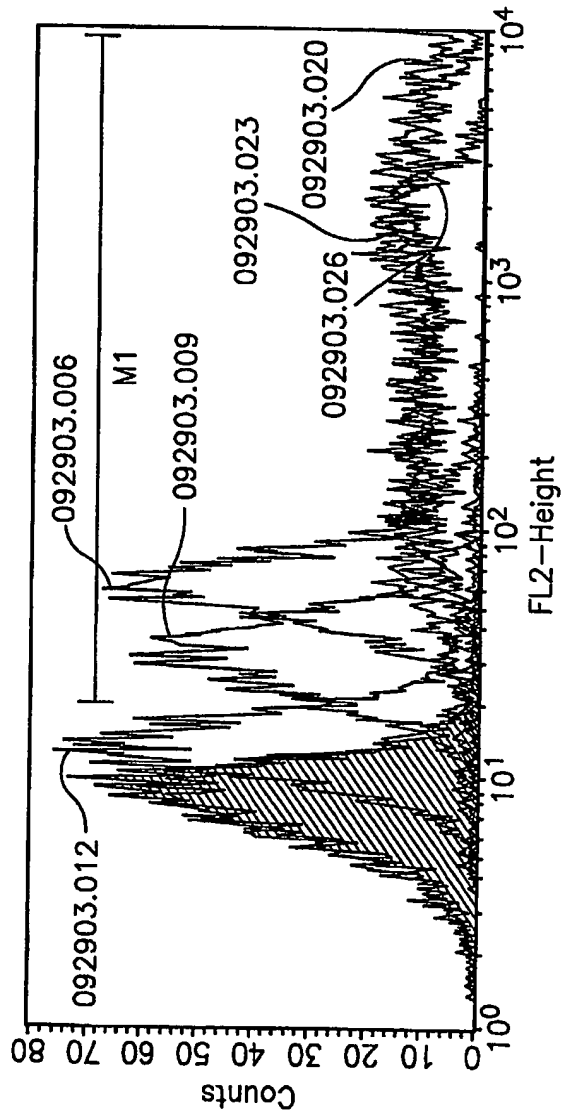
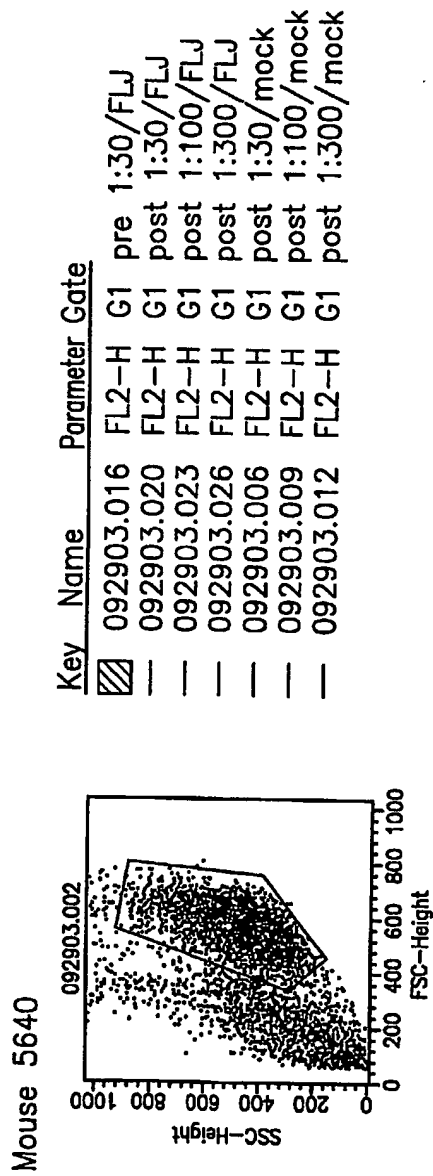
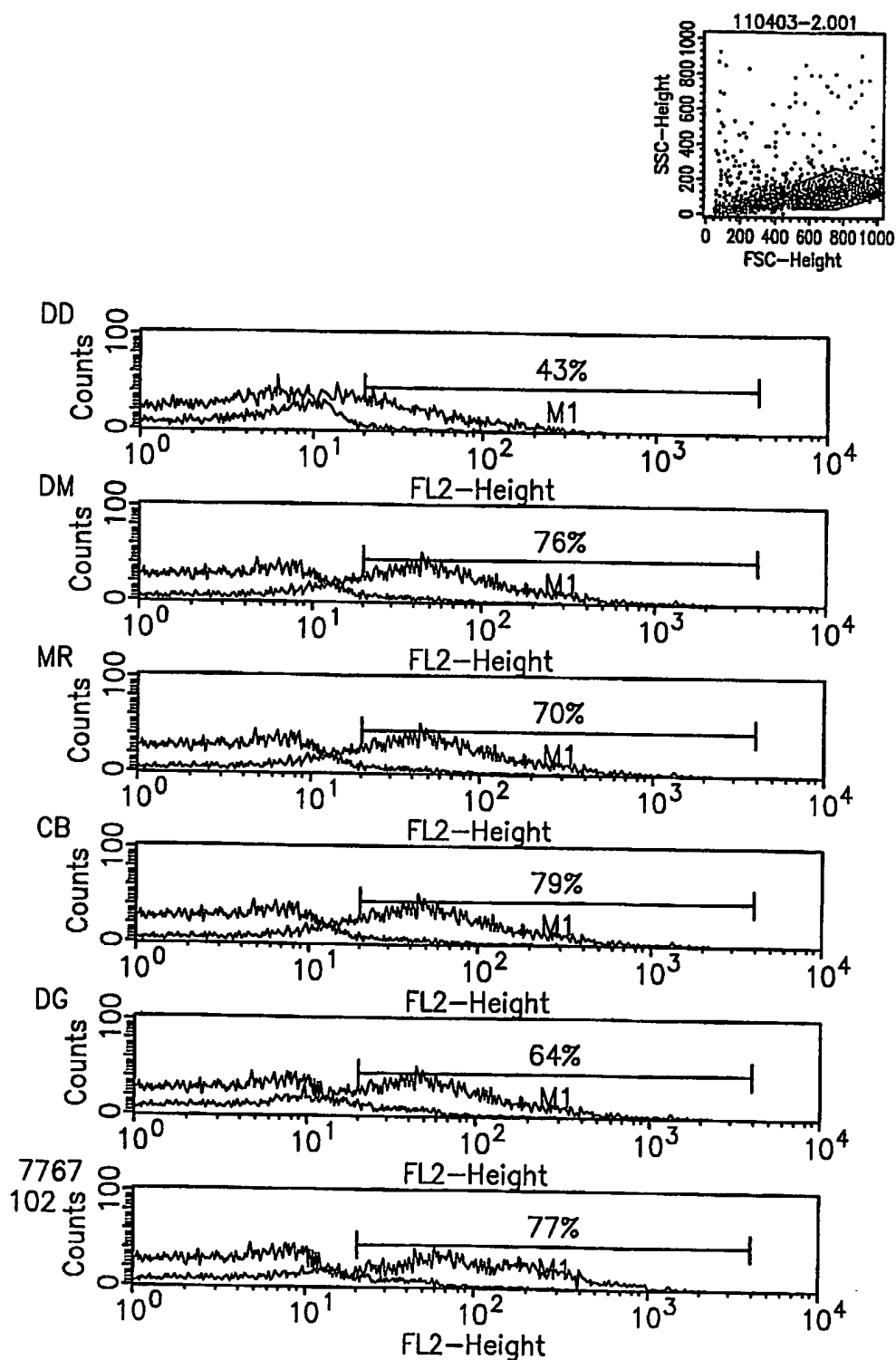


FIG. 10B

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**FIG. 11**

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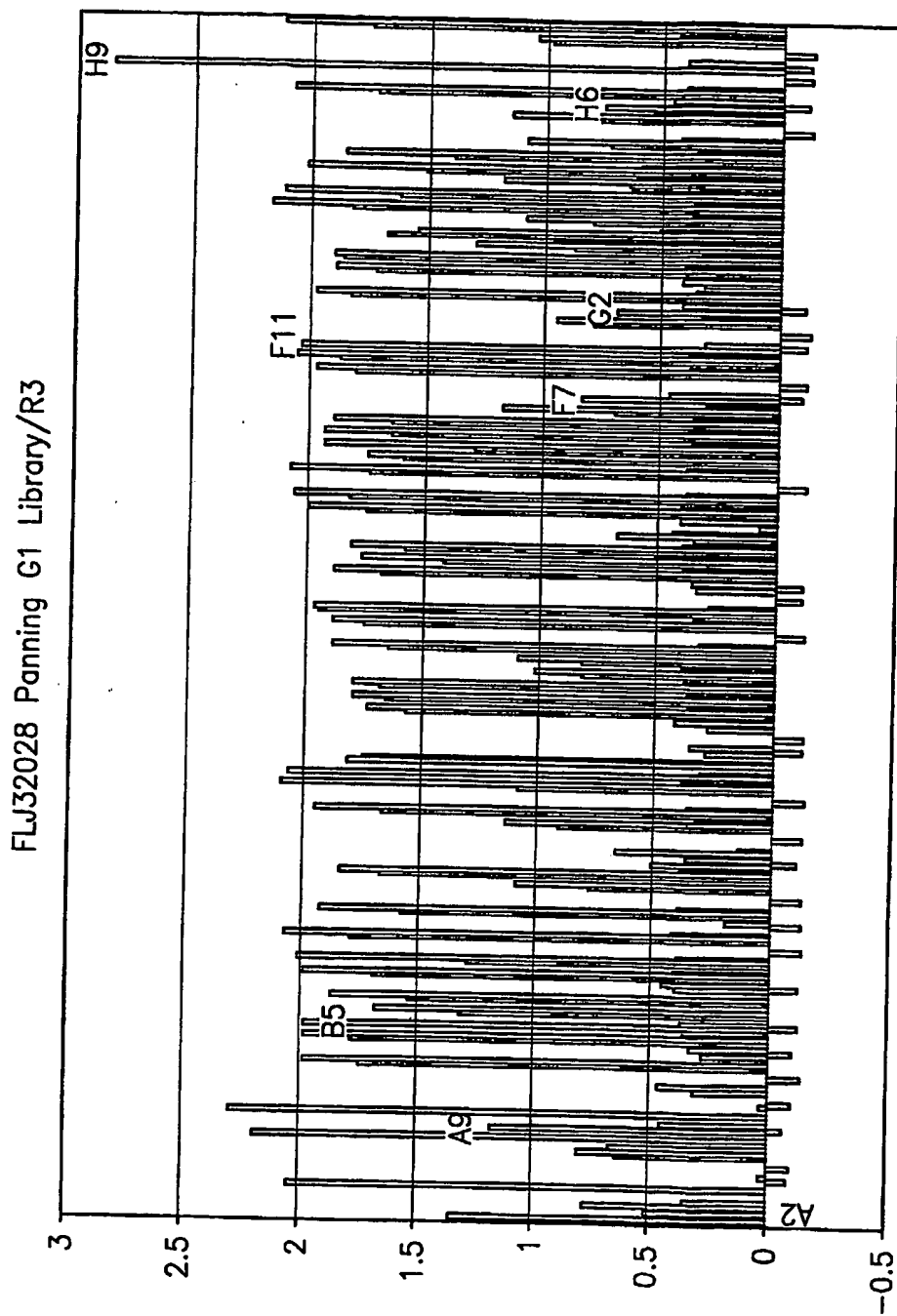


FIG. 12

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FIG. 13

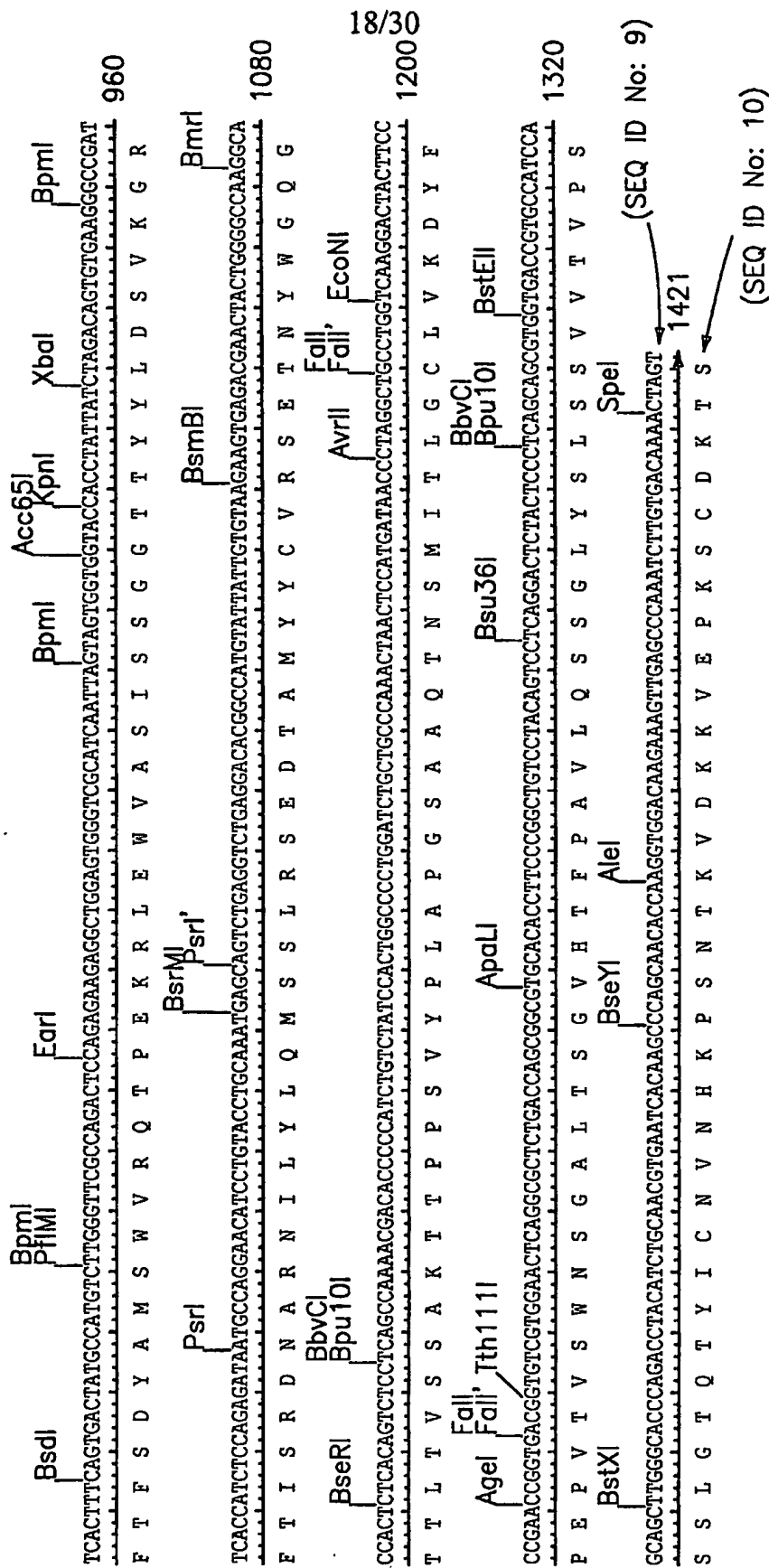
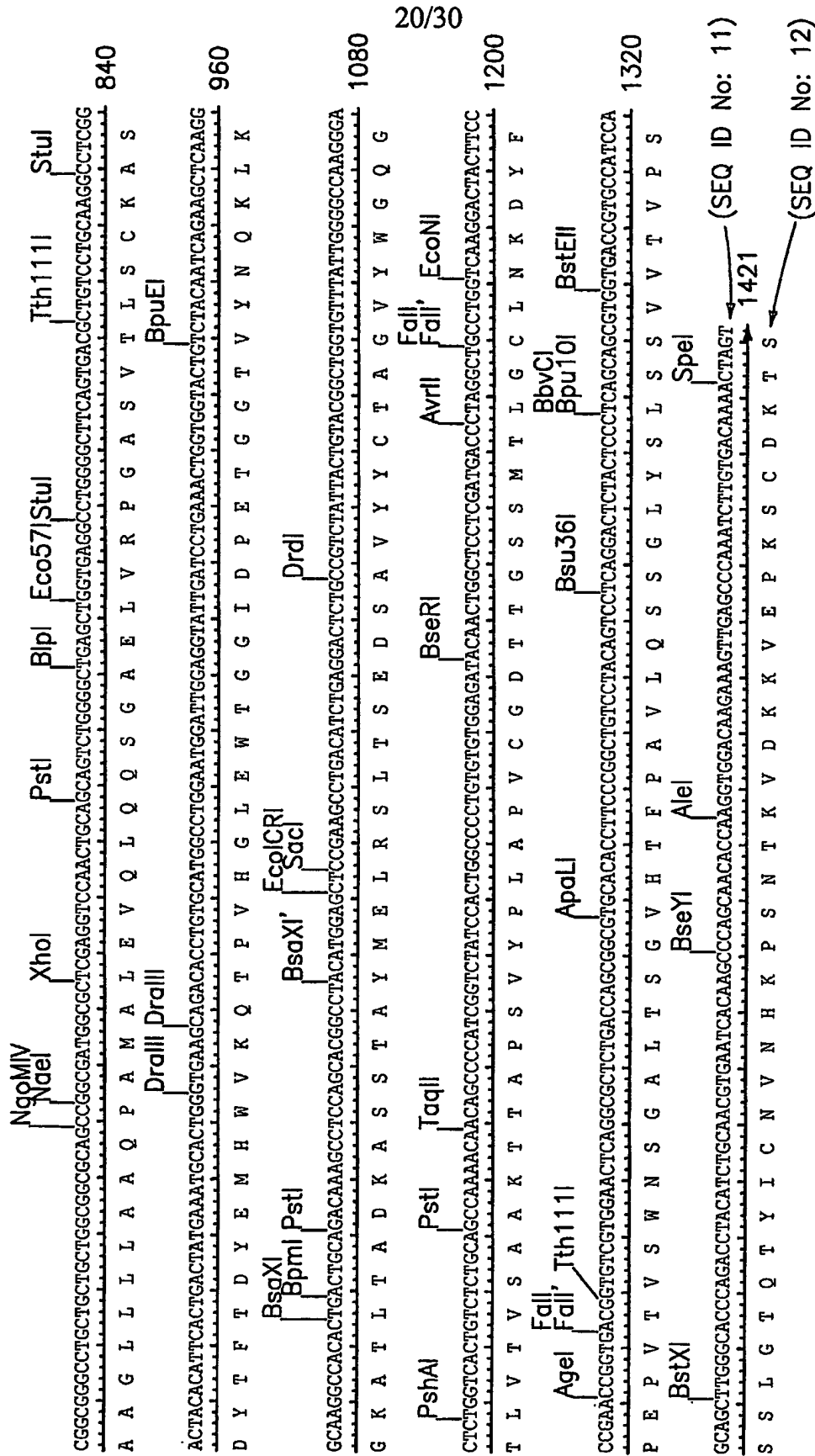


FIG. 13 (Cont.)

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FIG. 14



VL amino acid sequences of FLJ32028-specific IgG1 kappa clones from 5644 library

Clone	FR1	CDR1	FR2	CDR2
11F	DIQMTQTSSLSASLGDRVTISCRTS	QDISN-----Y LNWYQQKPDGTVKVLII	YTS	
11G	..V...AEL·SPVTS·ES·S...S·	KSLIYK-DGKT·	...L·R·GQSPQL...	FM·
6H	..V...SP...AV·V·EK·M...KS·	SLLYSSNQKN·	A·.....GQSP·L...	WA·
9H	..V·S·SP...AV·V·EK·M...KS·	SLLYSSNQKN·	A·.....GQSP·L...	WA·
9A	·VV...·PL·PV...·QAS...·S·	S·VHS-NGNT·	E·L...GQSP·L...	KV·
2G	·VV...·PL·PV...·QAS...·S·	S·VHS-NGNT·	E·L...GQSP·L...	KV·
7F	·VL...·PL·PV...·QAS...·S·	S·VHS-NGNT·	E·L...GQSP·L...	KV·

ELISA ODs:			
FR3	CDR3	FR4	FLJ/ Fc/ Fab
RLHSGVPSRFSGSGGTDSLTIINLEQEDIA	TYFC	QQGNTLPFTFGSG TKLEIKR	(SEQ ID NO: 13)
TRAP...SD...I...·FT·E·SRVKA·	·VGV·Y·	·LVEY·L...A·	(SEQ ID NO: 14)
TRE...D·T...·FT...·SSVKA·	·L·V·Y·	·YYSY·L...A·	(SEQ ID NO: 15)
ARG...D·T...·FT...·SSVKA·	·L·V·Y·	·YYSY·L·I·A·	(SEQ ID NO: 16)
NRF...D...·FT·K·SRV·A·	·LGV·Y·	F·SHV·L...A·	(SEQ ID NO: 17)
NRF...D...·FT·K·SRV·A·	·LGV·Y·	F·SHV·L...A·	(SEQ ID NO: 17)
NRF...D...·FT·K·SRV·A·	·LGV·Y·	F·SHV·L...A·	(SEQ ID NO: 17)

FR: framework region; CDR: complementarity determining region. Dots indicate identities with the clone 11F sequence. Dashes indicate gaps in the alignment. ELISA ODs are given for binding of the phage antibody clones to FLJ32028-Fc fusion protein(FLJ), to negative control Fc fusion protein(Fc), and to Anti-F(ab')₂ (Fab).

FIG. 15

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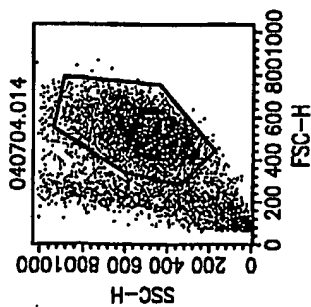
VH amino acid sequences of FLJ32028-specific IgG1 kappa
clones from 5644 library

Clone	FR1	CDR1	FR2	CDR2
11F	LEVQLQQSGAELVRPGASVTLCKAS	GYTFDYEMH WVKQTPVHGLEWIG	GIDPEIGGTVYNQKFKG	
11G	D.....L..
5BV.....Q..D..S..A.....
6HK.....K.....T..	..FNIK..TYINR.EQ.....	R..ANN..N..DP..Q..
9HF.....K.....TG..	..FNIK..TY..NI..R.EQ.....	M..AN..N..Q..DP..Q..
9A	..K..VE..GG..K..G..LK..A..	..F..S..A..S ..R..	..EKR...VA S	S-SG..T..Y..LDSV..
2G	..AK..VE..GG..K..G..LK..A..	..F..S..A..S ..R..	..EKR...VA S	S-SG..T..Y..LDSV..
7F	..K..VE..GG..K..G..LK..A..	..F..S..A..S ..R..	..EKR...VA S	S-SG..T..Y..LDSV..

ELISA ODs:			
FR3	CDR3	FR4	FLJ/ Fc/ Fab
KATLTADKSSGTAYMELRSLTSEDSAVYYCTS	F---AY WGQGLTVTSA		2.14/0.12/0.46 (SEQ ID NO: 18)
.....A..S.....A G---V.....		1.98/0.14/0.41 (SEQ ID NO: 19)
.....R.....A G---AD ..		1.84/0.11/0.53 (SEQ ID NO: 20)
...I...TP..N...LQ..S.....TD...V.. GGYFD..TL...S	0.86/0.12/0.61 (SEQ ID NO: 21)
...I...T..N...LQ..S.....T..... GGYFD..TL...S	2.95/0.11/0.56 (SEQ ID NO: 22)
RF..ISR..NARNIL..LQMS..R...T..M...VR	S-ETN..TL...S	1.30/0.17/0.61 (SEQ ID NO: 23)
RF..ISR..NARNIL..LQMS..R...T..M...VR	S-ETN..TL...S	0.80/0.12/0.57 (SEQ ID NO: 24)
RF..ISR..NARNIL..LQMS..R...T..M...VR	S-ETN..TL...S	0.95/0.13/0.62 (SEQ ID NO: 23)

FR: framework region; CDR: complementarity determining region. Dots indicate identities with the clone 11F sequence. Dashes indicate gaps in the alignment. ELISA ODs are given for binding of the phage antibody clones to FLJ32028-Fc fusion protein(FLJ), to negative control Fc fusion protein(Fc), and to Anti-F(ab')₂ (Fab).

FIG. 16



Key	Name	Parameter	Gat
▨	040704.014	FL2-H	G1 A
—	040704.022	FL2-H	G1 B
—	040704.020	FL2-H	G1 C
—	040704.024	FL2-H	G1 D
—	040704.019	FL2-H	G1 E

Key	Name	Parameter	Gat
▨	040704.014	FL2-H	G1 A
—	040704.018	FL2-H	G1 B
—	040704.021	FL2-H	G1 C
—	040704.017	FL2-H	G1 D
—	040704.023	FL2-H	G1 E

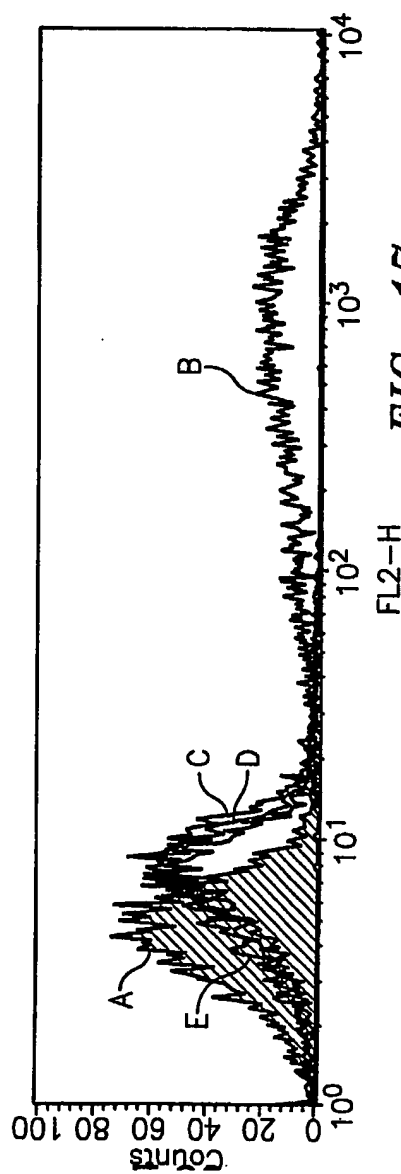
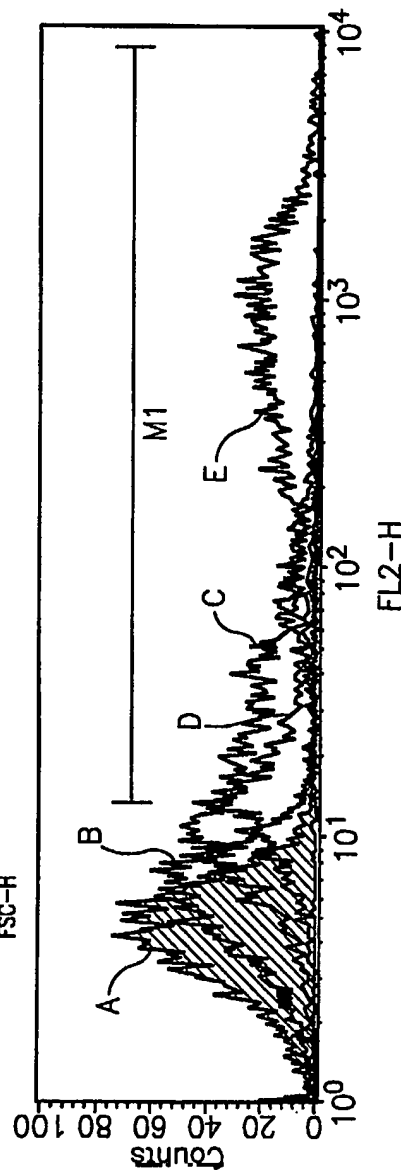


FIG. 17

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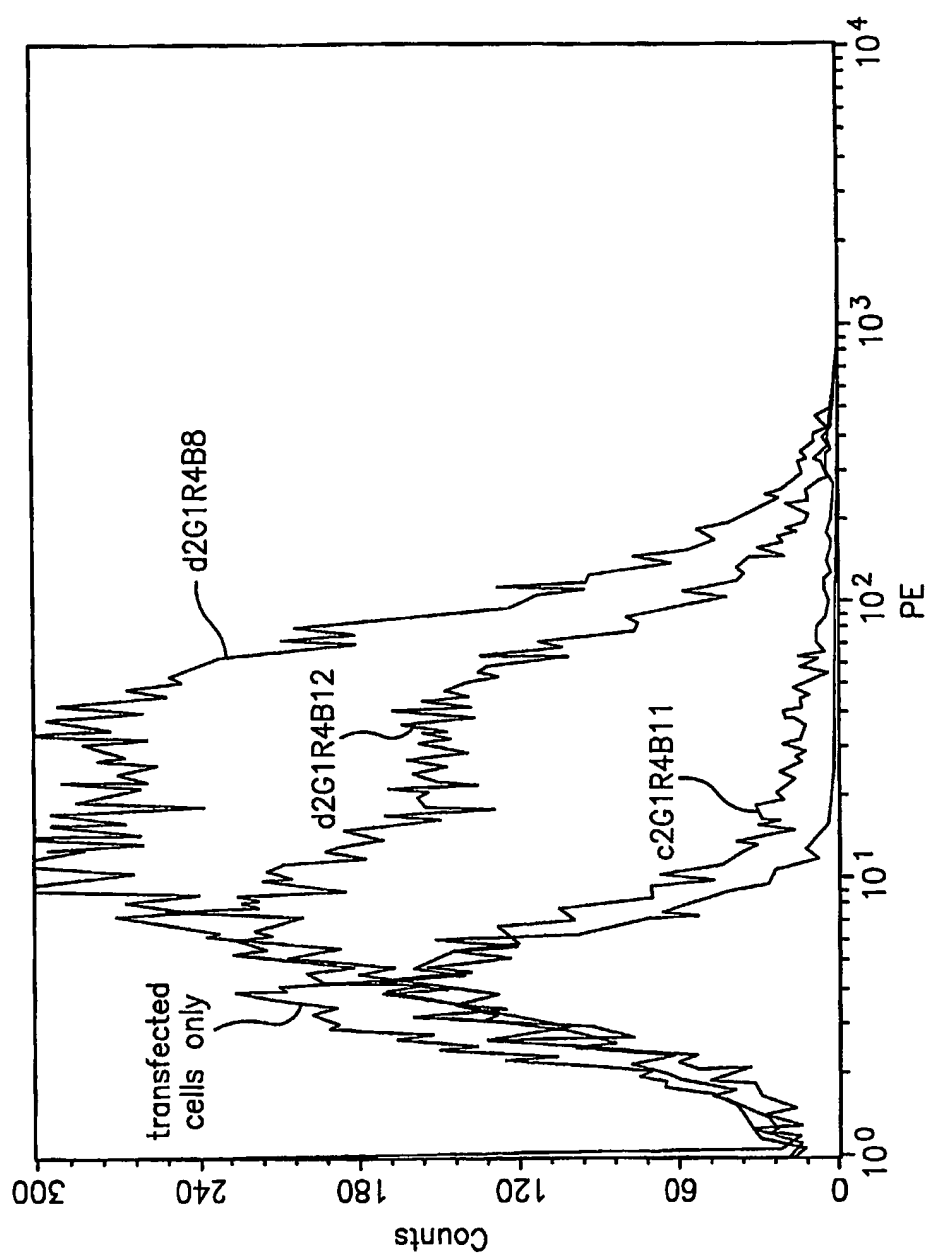


FIG. 18A

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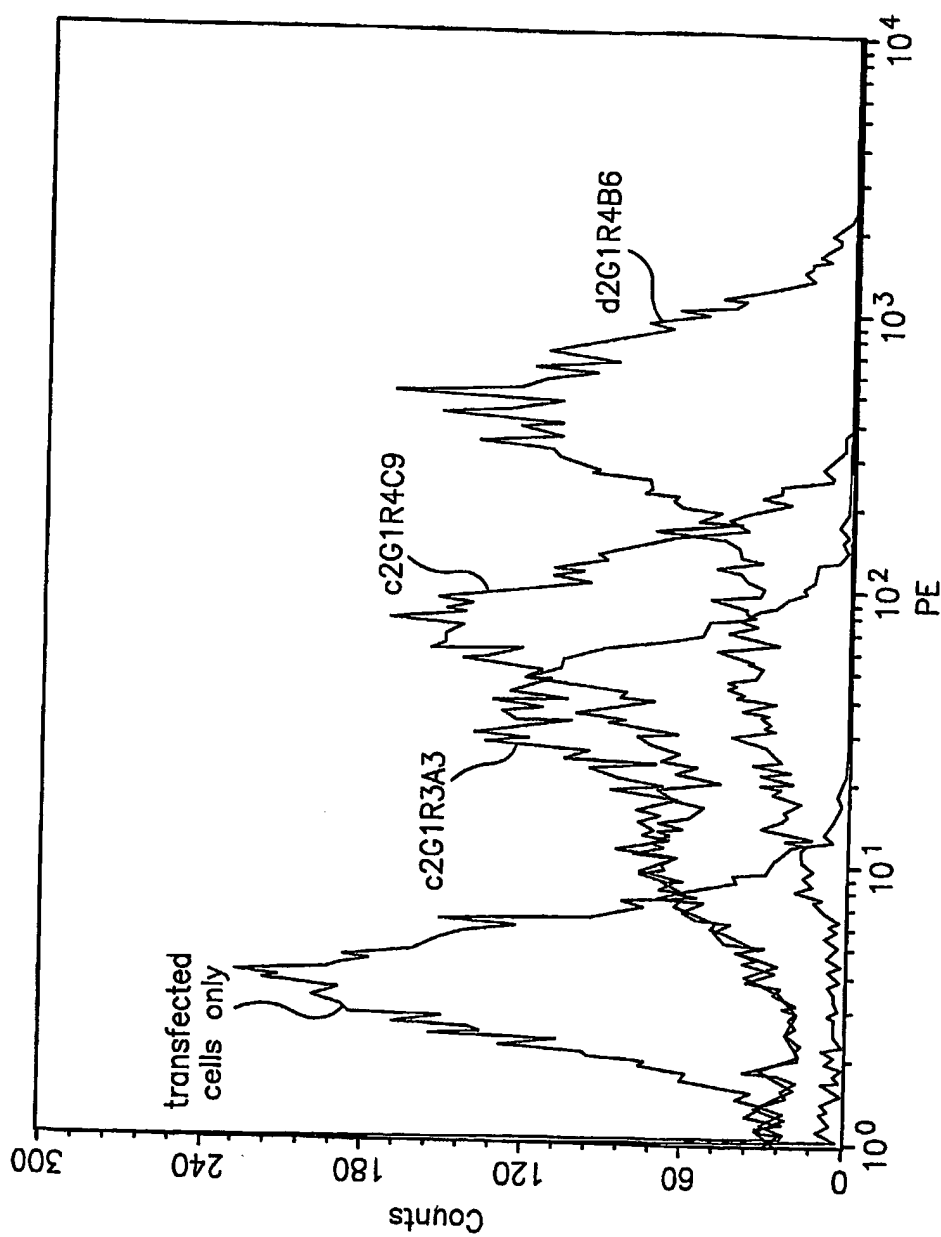
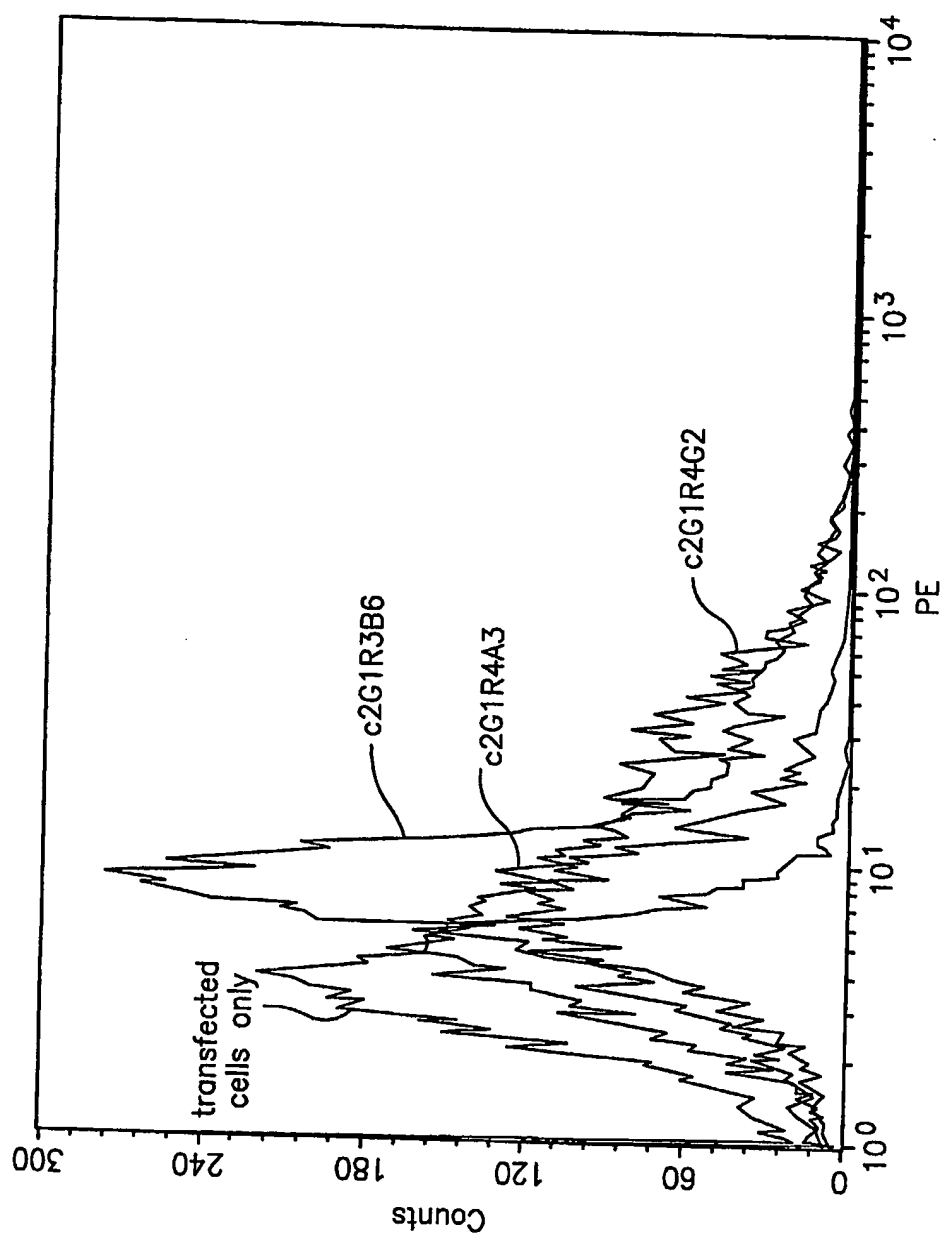


FIG. 18B

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*FIG. 18C*

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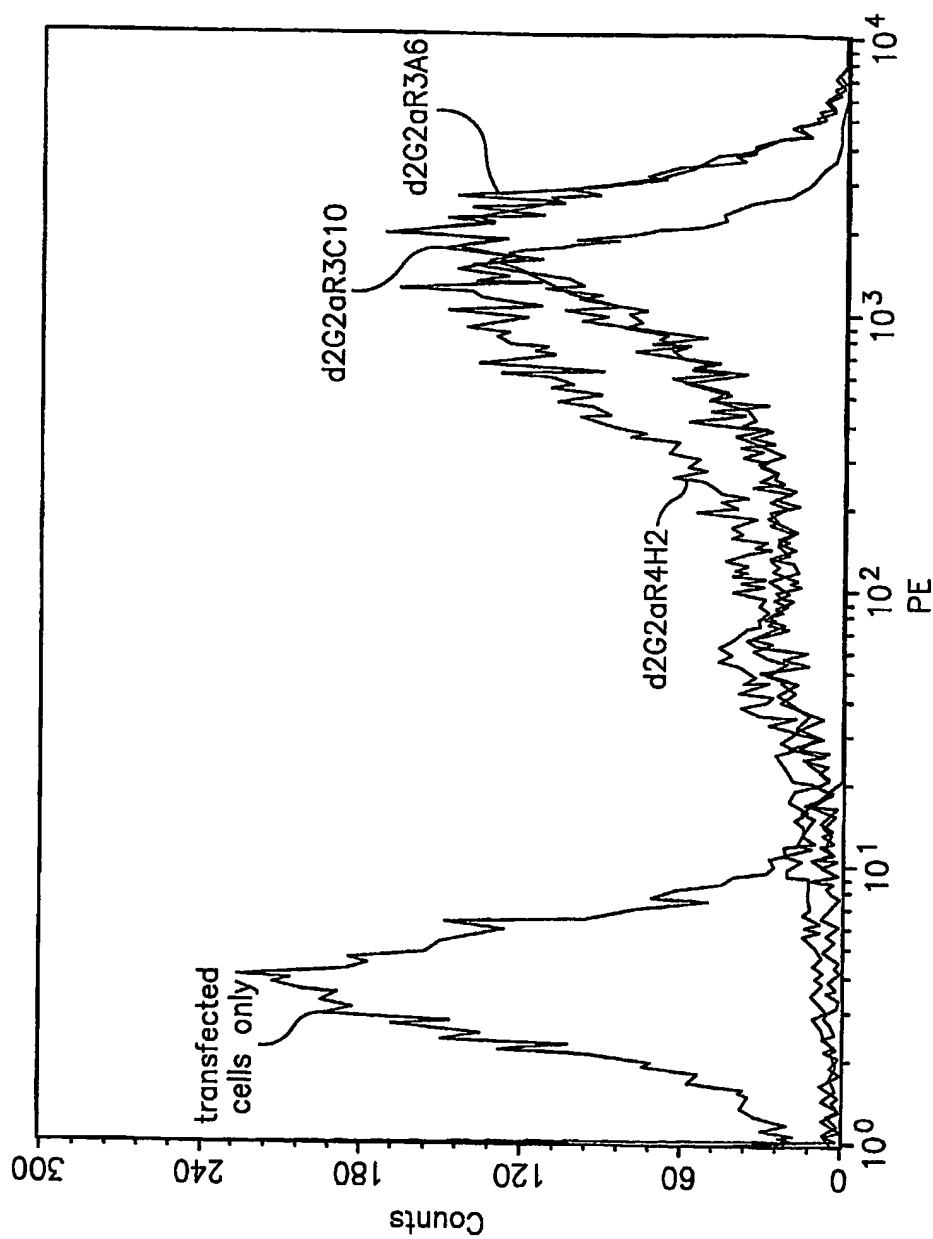
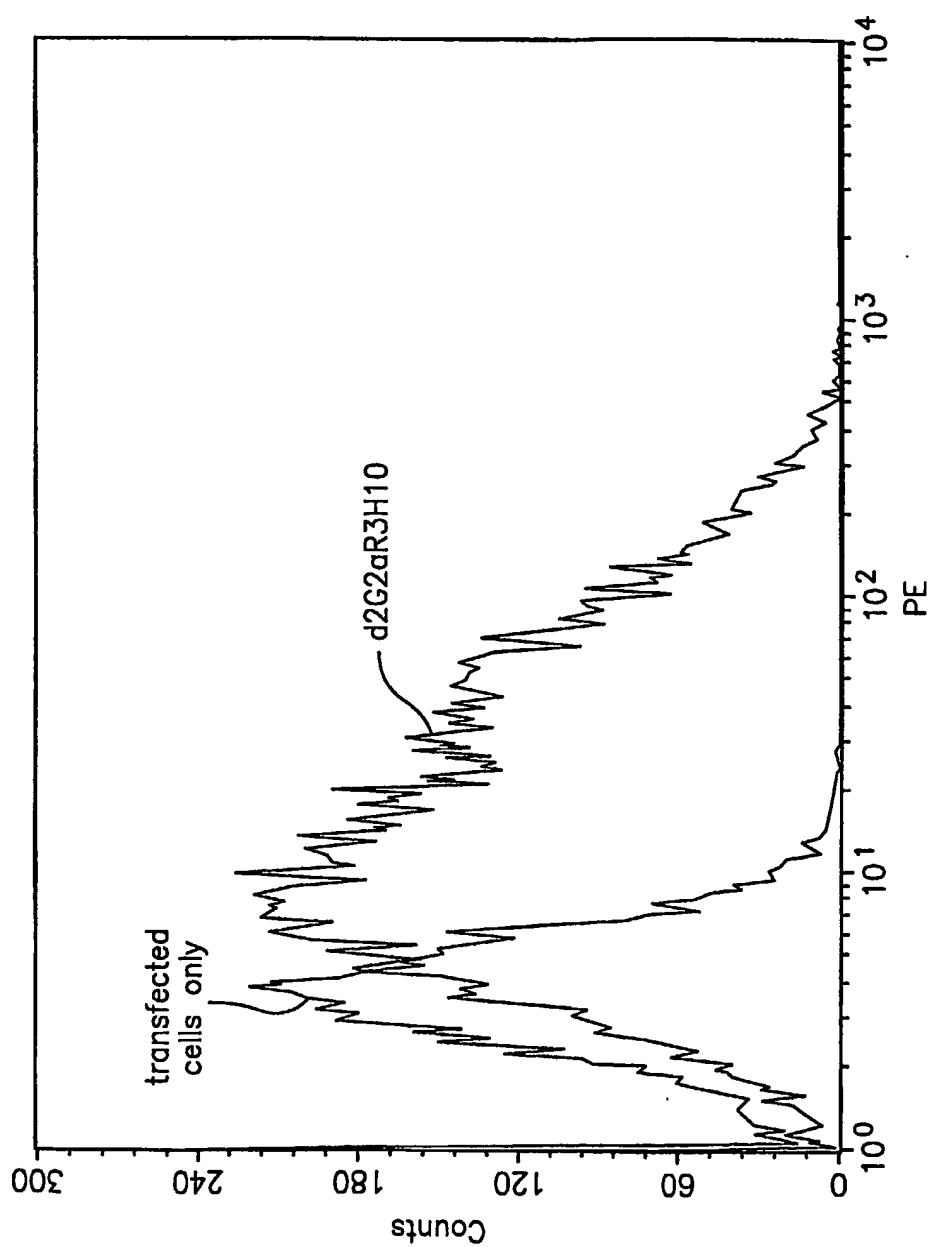


FIG. 18D

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*FIG. 18E*

FLJ32028 binding clones

Fab	FR1	CDR1	FR2	CDR2
C2G1R3A3	LEVQLVESGGGLVQPKGSLKLSCAAS	GFNFNTYAMN	WVRQSPGKGLEWVA	RIRTKSNNYATYYADSVKD
C2G1R3A12A.....	...S.....
C2G1R3C2A.....	...S.....
C2G1R3E3A.....	...S.....
C2G1R3F5	..K.....A.....	...S.....
C2G1R3F6	..K.....A.....	...S.....
d2G1R4B6A.....	...S.....
C2G1R4C9A.....	...S.....
C2G1R3B6	LEVQLQQSGAELAKPGASVKMSCKAS	GYFTNSWIH	WVKQRPQGLEWIG	YIHPGPGYTEYNQNFKD
C2G1R4G2	LEVQLKQSGAELVKPGASVKLSCTAS	GFNIKDTYMN	WVKQRPQGLEWIG	GIDPANDNTEYVVPKFQG
C2G1R4A3Q.....
d2G1R4B12	LEVQLQQSGAELVRPGASVTLCKAS	GYTFTDYEMH	WVKQTPVHGLEWIG	GIDPETGGTVYNQKFKG
d2G1R4B8
d2G2aR3A6	LEVQLVESGGGLVQPKGSLKLSCAAS	GFTFNTYAMN	WVRQAPGKGLEWVA	RIRTKSNNYATYYADSVKD
d2G2aR3C10
d2G2aR4A12
d2G2aR4G6
d2G2aR4H2
d2G2aR3H10	LEVQLQQSGAELVRPGASVTLCKAS	GYTFTDYEMH	WVKQTHVHGLEWIG	GIDPETGGTVYNQKFKG

FIG. 19

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Fab	ELISA/FACS				SEQ ID NO:
	FR3	CDR3	FR4	(geo mean)	
c2G1R3A3	RFSVSRDDSQSMLYLQMNLLKTEDTAMYYCVR	HEGDFAY	WGQGTLLTVSE	0.582/20.46	24
c2G1R3A12	..TI.....A	0.492/16.02	25
c2G1R3C2	..TI.....A	0.481/9.68	25
c2G1R3E3	..TI.....A	0.507/12.67	25
c2G1R3F5	..TI.....A	0.356/12.61	26
c2G1R3F6	..TI.....A	0.446/14.7	26
d2G1R4B6	..TI.....A	0.980/156	25
c2G1R4C9	..TI.....	..N....A	0.360/24.62	27
c2G1R3B6	KATLTADKSSSTAYIQLSSLTSEDSAVYYCIR	GGDWGY	WGQGTSLTVSS	1.184/10.6	28
c2G1R4G2	RATITADTSSNTAYLQLRSLTSDDTAVYYCVT	GGYFDY	WGQGTLLTVSS	2.412/11.08	29
c2G1R4A3	0.743/5.81	30
d2G1R4B12	KATLTADKSSSTAYMELRSQTSSEDSAVYYCTR	WDY	WGQGTLLTVSS	1.426/13.8	31
d2G1R4B8	1.415/17.84	31
d2G2aR3A6	RFTISRDDSQSMLYLQMNLLKTEDTATYYCVR	QGENRFAY	WGQGTLLTVSA	0.622/552.3	32
d2G2aR3C10	0.574/535	32
d2G2aR4A12	0.592/366	32
d2G2aR4G6	0.184/164	32
d2G2aR4H2	0.551/301.8	32
d2G2aR3H10	KATLTADKSSSTAYMELRSQTSSEDSAVYYCTS	SLP	WGQGTLLTVSA	0.740/16.41	33
negative control					

Top sequence in each group is used as a reference for others in the same group. Only the amino acids different from the reference sequence are shown in each sequence and the same amino acids are indicated by dots. ELISA is OD405 reading at 60 min to the FLJ32028 coated wells (4 µg/ml) and FACS (geo mean) is showing geometric mean.

FIG. 19 (Cont.)